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Dietary vitamin A requirement of juvenile Amur sturgeon (*Acipenser schrenckii*)

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Summary

The present experiment was conducted to determine the dietary vitamin A requirement of juvenile Amur sturgeon (*Acipenser schrenckii*) by formulating seven semipurified diets containing 10, 258, 510, 1050, 2020, 4100 and 8300 IU vitamin A (as retinol acetate) kg^{-1} diet, respectively. Each experimental diet was fed to triplicate groups of 20 juveniles each with initial average weights of 12.09 ± 0.22 g in 405-L aquaria and maintained at 25.0 ± 2.0°C for 8 weeks. Fish fed the basal diet (10 IU vitamin A kg^{-1} diet) exhibited poor appetite and activity, whereas these signs were not observed in any group fed vitamin A-supplemented diets. Weight gain, feed efficiency and hepatosomatic index increased significantly with increases in the dietary vitamin A level, reaching a peak with the vitamin A 1050 IU kg^{-1} diet, and then decreasing. Muscle chemical compositions were not affected by the dietary vitamin A levels. Vitamin A concentrations in liver and muscle increased significantly as the vitamin A levels increased within a range of 10∼4100 IU kg^{-1} diet; above this level there were no significant changes. Broken-line regression analysis of weight gain and liver vitamin A concentration against the dietary vitamin A level showed that juvenile Amur sturgeon required a minimum of 923 IU vitamin A kg^{-1} in the diet for maximal growth, and 1981 IU kg^{-1} for highest liver vitamin A accumulation.

Introduction

Vitamin A has the distinction of being the first fat-soluble vitamin to be recognized. It is certainly one of the most versatile vitamins, with roles in such diverse functions as vision, immune defenses, maintenance of body linings and skin, bone and body growth, normal cell development, and reproduction. Fish lack the capacity for vitamin A synthesis and therefore must obtain it from the diet (Halver, 2002); the normal range requirement reported for most fish species is 1000–20 000 IU kg^{-1} diet (NRC, 1993). Vitamin A affected the survival and growth rates of Siberian sturgeon larvae and high dietary levels of vitamin A appeared to be detrimental, thus the requirement was not recommended (Stéphanie et al., 2006). All vertebrate fish may suffer from vitamin A deficiency and/or toxicity, and the final biological outcome of both deficiency and toxicity are very similar in most species; these include mortality, reduced growth, impaired skeletal formation, blindness, reduced mucous secretion and haemorrhaging of eyes, fins and skin (Halver, 1989; Takeuchi et al., 1998; Cuesta et al., 2002).

Acipenser schrenckii is a large riverine sturgeon species native to the Amur River. The species readily adapts to culture conditions, including traditional Chinese fish culture ponds, lakes, reservoirs, and cages. It reveals good growth performance on a variety of feeds, with 1-year-old fish reared on commercial diets attaining 900–1200 g. Amur sturgeon has become the most popular sturgeon species for aquaculture in China (CITES, 2000; Zhuang et al., 2002; Wei et al., 2004). Some studies have been conducted on the species requirement for fat and protein as well as its effects on digestibility (Xiao and Lin, 2001; Li et al., 2002). So far, little information is available on nutrient requirements of different species of sturgeon (Hung and Deng, 2002). Only four vitamins have been studied in sturgeon: ascorbic acid, choline, vitamin E and vitamin A (Hung, 1989; Falahatkar et al., 2006; Stéphanie et al., 2006). However, none of them addressed the vitamin requirements of Amur sturgeon. Thus the purpose of this study was to estimate the dietary vitamin A requirement of Amur sturgeon juveniles.

Materials and methods

Diets

Composition of the basal diet is given in Table 1. Protein and lipid sources were casein, gelatin, soybean meal, soybean oil and maize oil. Vitamin A amounts added as retinyl acetate (Rovimix A500R, Roche) were 0, 250, 500, 1000, 2000, 4000, and 8000 IU kg^{-1} diet (dry weight), respectively. The analyzed

Table 1
Ingredient composition and proximate analysis of the basal diet in *Acipenser schrenckii*

Ingredients	g kg^{-1}	Nutriments	%
Casein	360	Moisture	13.2
Gelatin	60	Crude protein	41.4
Dextrin	200	Ash	4.4
Soybean meal	200	Crude fiber	7.0
Soybean oil	50	Crude lipid	8.8
Maize oil	50		
Vitamin mixture[a]	7.5		
Mineral mixtrue[b]	30		
Choline chloride	2.5		
Micro-cellulose	40		

[a]Vitamin premix supplied the diet with (mg kg^{-1} dry diet): thiamine, 50; riboflavin, 200; pyridoxine HCl, 50; Ca-pantothenate, 400; nicotinic acid, 750; folic acid, 15; cyanocobalamin, 0.1; biotin, 5; inositol, 2000; ascorbic acid, 325, cholicalciferol, 1; DL-α-tocopheryl acetate, 200; menadione, 40.
[b]Mineral premix supplied the diet with (mg kg^{-1} dry diet): $ZnSO_4.7H_2O$, 350; $MnSO_4.4H_2O$, 40; $CoCl_2.6H_2O$, 80; $CuSO_4.5H_2O$, 12; $AlCl_3.6H_2O$, 15; KIO_3, 5; $FeSO_4.7H_2O$, 1000; NaCl, 5000; Na_2SeO_3, 6; $Ca(H_2PO_4)_2.H_2O$, 15 000.

dietary vitamin A concentrations of the seven diets estimated by HPLC (Driskell et al., 1982) were 10 (unsupplemented basal diet), 258, 510, 1050, 2020, 4100, and 8300 IU kg^{-1} diet. Vitamin A was added to the basal diet at the expense of cellulose. The ingredients were blended and then extruded through a mincer with a 2-mm eyelet die. The moist pellets were dried in a forced air oven at room temperature for 2 h and then stored at −20°C protected from light until used.

Experimental fish and husbandry

Early juvenile Amur sturgeon were obtained from the Chinese Sturgeon Breeding Base of the Yangtze River Fisheries Research Institute, CAFS (Jingzhou, Hubei, China) and conditioned for 2 weeks during which they were fed the basal diet to reduce body storage of vitamin A. After acclimatization, groups of 20 randomly selected fish (12.09 ± 0.22 g) were stocked into 21 405-L flow-through aquaria. The aquaria were filled with 315-L filtrated lake water with continuous aeration. The flow rate was set at approximate 600 ml min^{-1}. Water quality was monitored daily. Water temperature was maintained at 25.0 ± 2.0°C, dissolved oxygen concentrations were >6.0 mg L^{-1}, ammonia–nitrogen concentrations were <0.22 mg L^{-1}, and nitrite–nitrogen did not exceed 0.05 mg L^{-1} at any point during the acclimation period or the feeding trial. Fish were fed their respective diets at a rate about 3% (weeks 1–4) and 2% (weeks 5–8) of their body weight four times per day (07 : 00, 12 : 30, 18 : 00, and 22 : 30 h) during the 8-week experiment. The juveniles were weighed every 14 days and the ration was adjusted accordingly. Feed not consumed was removed 45 min after feeding and quantified for measurements of feed intake; fecal matter was then siphoned from the bottom of each aquarium.

Analytical methods

Diets were analyzed for crude protein, crude lipid, moisture and ash according to the AOAC (1985).

At the beginning of the feeding study, 20 fish in each aquarium were individually weighed after starvation for 24 h. Final weight was determined at the end of the study 24 h after the last feeding. Three fish from each aquarium were collected and frozen. The samples were later freeze-dried, ground and analyzed for protein and fat composition using the same method as in the basal diet. At the same time, liver and muscle tissue were extracted from four fish from each aquarium for analysis. Vitamin A concentration therein were determined using the HPLC method proposed by He et al. (2004) with the following modifications: A weighed portion of fish liver and muscle (about 8 g) was triturated with a glass homogenizer in an ice bath and poured into a 250 ml bunsen flask; 100 ml of pyrogallic acid ethanol solution (15 g ml^{-1}) was added, followed by 50 ml arsonium hydroxide solution (500 g L^{-1}). The mixture was placed in a boiling water bath for 30 min. After cooling, it was extracted three times with 100 ml ligarine in a tap funnel. The extract was incorporated and washed by deionized water to neutrality, then dessicated by anhydrous sodium sulfate. Subsequently, the preparation was concentrated in a rotary evaporator (Buchi R210) and the remains dissolved using 2 ml chromatographic grade carbinol. Lastly, the carbinol solution was filtrated and the vitamin A contents determined by HPLC. The system consisted of a pump (Waters 515), autosampler (Waters, 717), UV-detector (Waters, 2487) and a C18, 4.6 × 250 mm column (Symmetry). The mobile phase was 8% solution of tert-butyl methyl ether in *N*-heptane, and the quantification of ROH was carried out using an external standard curve.

Statistical analysis

Statistical tests were performed using the CSS package: Statistica TM, Release 6.0 (Statsoft, USA). Results were analyzed by one-way analysis of variance (ANOVA).When the ANOVA identified differences among groups, multiple comparisons among means were made with Duncan's new multiple range test. Statistical significance was determined by setting aggregate type I error at 5% ($P < 0.05$) for each set of comparisons. Dietary vitamin A requirement for juvenile Amur sturgeon was estimated by the broken-line regression method (Robbins et al., 1979).

Results

Survival and growth performance

After the 8-week feeding trial, all groups revealed a 100% survival rate. Fish fed the basal diet (10 IU vitamin A kg^{-1} diet) exhibited lack of appetite and stolidity of activity. However, no such signs were observed in any group fed the vitamin A-supplemented diets.

Dietary vitamin A supplementation significantly improved growth of juvenile Amur sturgeon. Weight gain (WG) of the fish fed the basal diet was significantly lower than that of the other groups, and the fish fed A_4 (1050 IU vitamin A kg^{-1}) showed a significant higher WG than those fed A_1, A_2, A_3, A_6 and A_7 diets ($P < 0.05$). However, there was no significant difference in WG between fish fed A_2 and A_3 diets, A_4 and A_5 diets, and among fish fed A_5, A_6, and A_7 diets (Table 2). The pattern of difference in feed efficiency was similar to the findings of weight gain. The control group A_1 had the lowest hepatosomatic index, which was significantly lower than the other groups ($P < 0.05$). Groups A_4 and A_5 had the significantly highest hepatosomatic index of 3.03% and 2.84%, respectively ($P < 0.05$). However, the hepatosomatic indexes for A_2, A_3, A_6 and A_7 were not significantly different ($P > 0.05$). The condition factor of the control group A_1 was significantly lower than those of all the other groups ($P < 0.05$), but among the later, there was no significant difference.

By broken-line regression analysis (Robbins et al., 1979), the relation between the weight gain and dietary vitamin A concentration could be denoted by the following equations:

$$y = 0.1681X + 434.98 (r^2 = 0.86)$$

$$y = -0.0028X + 592.72 (r^2 = 0.87)$$

The intersected point of these two lines represented that the lowest dietary vitamin A concentration was 923 IU kg^{-1} for maximal weight gain.

Muscle composition

Addition of vitamin A to the basal diet decreased muscle fat concentration. Fish fed the basal diet showed higher muscle fat concentration than fish fed the diets containing vitamin A. Muscle fat was the minimum at 1050 IU vitamin A kg^{-1} (A_4), but there was no statistically significant difference among treatments. In addition, vitamin A supplementation to the

Table 2
Growth performance (means ± SD, n = 3) of juvenile Amur sturgeon (*Acipenser schrenckii*) fed experimental diets for 8 weeks. Values are means of three groups of fish (n = 3) with 20 fish per group. Within a row, values with different superscript indicates significant differences at a level of P < 0.05, analyzed by means of ANOVA

Vitamin A (IU kg^{-1})	Initial weight (g)	Final weight (g)	WG[a] (%)	FE[b] (%)	HSI[c] (%)	CF[d] (%)
A_1 10	12.12 ± 0.27	60.36 ± 3.32^{a}	417.40 ± 34.92^{a}	95.03 ± 2.35^{a}	2.15 ± 0.09^{a}	0.45 ± 0.04^{a}
A_2 258	12.05 ± 0.45	70.11 ± 2.94^{a}	494.16 ± 26.34^{b}	108.11 ± 2.65^{b}	2.46 ± 0.06^{b}	0.66 ± 0.05^{b}
A_3 510	11.83 ± 0.08	75.31 ± 4.13^{a}	534.61 ± 23.45^{c}	108.52 ± 1.38^{b}	2.56 ± 0.13^{b}	0.68 ± 0.01^{b}
A_4 1050	12.05 ± 0.30	83.54 ± 1.76^{b}	601.02 ± 20.40^{d}	115.29 ± 2.45^{c}	3.03 ± 0.27^{c}	0.71 ± 0.09^{b}
A_5 2020	12.07 ± 0.27	82.82 ± 1.94^{bc}	588.20 ± 14.23^{d}	110.77 ± 2.04^{c}	2.84 ± 0.17^{c}	0.71 ± 0.07^{b}
A_6 4100	12.00 ± 0.10	82.39 ± 2.30^{c}	576.22 ± 4.88^{d}	110.15 ± 3.30^{c}	2.54 ± 0.11^{b}	0.69 ± 0.01^{b}
A_7 8300	12.42 ± 0.18	83.24 ± 1.15^{b}	570.39 ± 9.21^{cd}	109.47 ± 2.11^{b}	2.45 ± 0.18^{b}	0.68 ± 0.06^{b}

[a]WG, weight gain = 100 × (final body weight − initial body weight)/initial body weight.
[b]FE, feed efficiency = 100 × (final body weight − initial body weight)/dry feed intake.
[c]HSI, hepatosomatic index = 100 × liver weight/body weight.
[d]CF, condition factor = 100 × (body weight, g)/(body length, cm)^3.

basal diet did not significantly affect muscle protein and moisture of the fish (Table 3).

Vitamin A concentrations in liver and muscle

Vitamin A concentrations in the fish liver and muscles were positively correlated with dietary levels of vitamin A (Figs 1, 2). Vitamin A levels in fish liver (5.16–89.20 μg g^{-1}) were much higher than those in fish muscle (0.37–7.09 μg g^{-1}). Fish fed diets exceeding 2020 IU kg^{-1} dietary vitamin A had significantly higher vitamin A concentrations in liver and muscle than those fish fed diets with lower concentrations (P < 0.05). By broken-line regression analysis, the relation between vitamin A contents in diets and its accumulation in fish muscles could be denoted in the following equation: $y = 0.0029\ x + 1.298$ ($r^2 = 0.91$); $y_{max} = 7.05$. The relation between vitamin A content in diets and its accumulation in livers could be found as follows: $y = 0.0388\ x + 12.19$ ($r^2 = 0.97$); $y_{max} = 88.99$. Estimated by the broken-line regression, the optimal dietary vitamin A level based on muscle and liver vitamin A concentrations was 1959 IU kg^{-1} and 1981 IU kg^{-1}, respectively.

Table 3
Effects of dietary vitamin A on muscle composition of juvenile Amur sturgeon (*Acipenser schrenckii*) fed experimental diets for 8 weeks

Vitamin A (IU kg^{-1})	Moisture (%)	Crude fat (%)	Crude protein (%)
A_1 10	78.68 ± 0.54	5.83 ± 0.37	16.88 ± 0.33
A_2 258	78.33 ± 0.58	5.77 ± 0.19	16.92 ± 0.21
A_3 510	78.06 ± 0.91	5.24 ± 0.44	16.98 ± 0.17
A_4 1050	78.67 ± 0.47	4.56 ± 0.63	16.84 ± 0.31
A_5 2020	78.69 ± 0.26	5.03 ± 0.39	17.12 ± 0.26
A_6 4100	78.35 ± 0.58	5.46 ± 0.76	17.01 ± 0.32
A_7 8300	79.07 ± 0.55	5.47 ± 0.46	16.75 ± 0.23

Values are means of three groups of fish with 20 fish per group (freeze-dried samples of three fish per group).

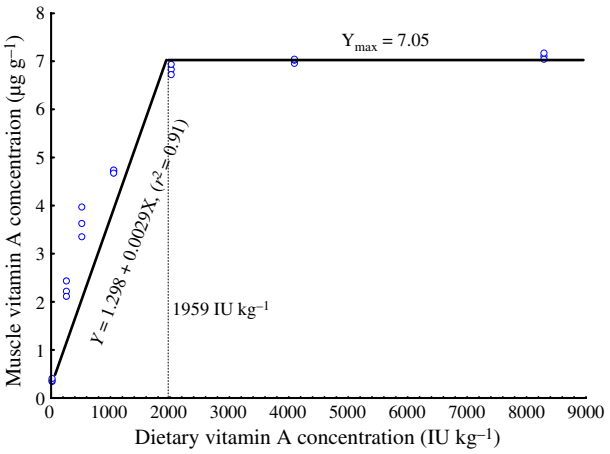

Fig. 1. Effects of dietary vitamin A on muscle vitamin A in Amur sturgeon (*Acipenser schrenckii*) fed experimental diets for 8 weeks. Data represent mean individual values of three replicates with an initial stocking density of 20 fish per group (four individuals per replicate taken at the end of feeding period)

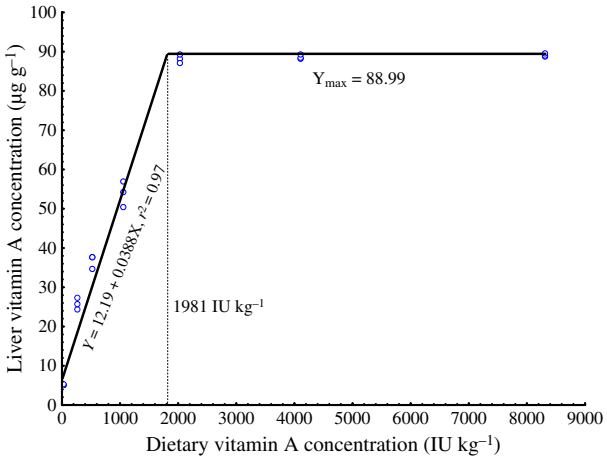

Fig. 2. Effects of dietary vitamin A on liver vitamin A in Amur sturgeon (*Acipenser schrenckii*) fed experimental diets for 8 weeks. Data represent mean individual values of three replicates with an initial stocking density of 20 fish per group (four individuals per replicate taken at the end of feeding period)

Discussion

This study indicated that a dietary source of vitamin A was essential for juvenile Amur sturgeon. In the present study, optimal dietary vitamin A requirement for maximum growth performance was 913 IU kg^{-1}, which was lower than those reported in several fish species, such as common carp (4000 IU kg^{-1}, Aoe et al., 1968; Suhenda and Djajadiredja, 1985), channel catfish and sea bream (2000–2500 IU kg^{-1},

Halver, 1989), guppy greasy grouper (3101 IU kg^{-1}, Mohamed et al., 2003), Atlantic halibut (8333 IU kg^{-1}, Moren et al., 2004), and hybrid tilapia (5850 IU kg^{-1}, Hu et al., 2006). Compared with these findings, the present results showed that Amur sturgeon seemed to be very susceptible to variations in dietary vitamin A.

Vitamin A deficiency is characterized by abnormal bone formation, exophthalmia, haemorrhage in the anterior eye chamber, night blindness, poor growth and vision and retinization of epithelial tissue (Halver, 1989). In the present study, fish fed only the basal diet exhibited bad appetite, stolid activity, and poor growth. However, the experiment was probably not of sufficient length to deplete tissue vitamin A concentration in order to produce signs of vitamin A deficiency. The negative effects of high levels of vitamin A, such as retarded growth (Mohamed et al., 2003; Hemre et al., 2004; Hernandez et al., 2005) and skeletal deformities (Hilton, 1983; Saleh et al., 1995) have been reported previously. However, the results in the present study showed that excessive vitamin A intakes by Amur sturgeons only reduced the fish growth performance in gross observation. The high vitamin A levels may become toxic over a long period of feeding. Because vitamin A toxicity in general is dependent on two factors: the level in the diet and the length of the feeding period (Moren et al., 2004). Thus, further research is needed to focus on vitamin A toxicity of Amur sturgeon over a long period.

The effects of various levels of vitamin A on the body composition of fish differed. Thompson et al. (1995) reported that the dietary vitamin A level did not influence the body composition of rainbow trout, whereas Mohamed et al. (2003) indicated a significant decrease in body fat along with the increase in the dietary vitamin A level. Furthermore, Hu et al. (2006) reported that dietary vitamin A had some influence on the body moisture and protein of hybrid tilapia. In the present study, a consistent trend for the effect of dietary vitamin A on muscle fat could not be identified.

In the present study, dietary vitamin A was also found to have positive effects on its accumulation in liver and muscle, similar to the report by Stéphanie et al. (2006). Vitamin A retention in liver did not increase significantly at dietary supplementation levels above 2020 IU kg^{-1}. Similar phenomena were reported for many fish species (Hernandez et al., 2004; Hu et al., 2006). This result indicated that at high dietary vitamin A concentrations, unutilized vitamin A was stored in liver, resulting in a significant increase of vitamin A retention.

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